Original Paper

VAP-1, a Novel Molecule Linked to Endothelial Damage and Kidney Function in Kidney Allograft Recipients

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Key Words

Kidney transplantation • Endothelium • VAP-1 • Renalase • Kidney function

Abstract

Background/Aims: VAP-1 (vascular adhesion protein-1) is a copper-containing SSAO (semi-carbazide sensitive amine oxidase) secreted by vascular smooth muscle cells, adipocytes, endothelial cells with functional monoamine oxidase activity. The oxidation process generates harmful products that may be involved in atherosclerosis and vascular damage. Elevation of SSAO activity is observed in atherosclerosis, diabetes mellitus and obesity. On the other hand, renalase, with possible monoamine oxidase activity, which breaks down catecholamines like SSAO, is also expressed in the endothelium as well as in the kidney. The aim of the study was to assess VAP-1 levels and its correlations with endothelial injury markers and renalase in 50 kidney allograft recipients. **Methods:** Hemoglobin, urea, creatinine, rate were studied by standard laboratory method in the hospital central laboratory. We assessed markers of endothelial function/injury: vWF, thrombomodulin, ICAM, VCAM, CD40L, CD44, CD146, inflammation: hsCRP, and IL-6 and adipocytokines: leptin, adiponectin, visfatin, apelin with commercially available assays. **Results:** The mean serum VAP-1 in Tx was significantly higher comparing to the control group. In kidney transplant recipients VAP-1 correlated with BMI ($r=0.39$, $p<0.01$), CD44 ($r=0.27$, $p<0.05$), hsCRP ($r=0.28$, $p<0.05$), serum creatinine ($r=0.29$, $p<0.05$), eGFR (CKD-EPI formula $r=-0.27$, $p<0.05$, MDRD $r=-0.27$, $p<0.05$, Cockcroft-Gault $r=-0.35$, $p<0.01$), serum urea ($r=0.27$, $p<0.05$), CD146 ($r=0.49$, $p<0.001$), CD40L ($r=0.26$, $p<0.06$), and renalase ($r=0.34$, $p<0.05$). In multiple regression analysis VAP-1 was predicted 80% by serum creatinine (beta value 0.33, $p=0.01$), and CD146 (beta value 43, $p=0.0005$). **Conclusion:** VAP-1, elevated in kidney transplant recipients, is predominantly dependent on endothelial damage and kidney function, which deteriorated with time after kidney transplantation.

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Introduction

The plasma-specific enzyme SSAO (semi-carbazine sensitive amine oxidase) catalyses the oxidative deamination of primary amines (methylamine, aminoacetone, benzylamine, 2-phenylamine are preferential and physiological substrates) to form the corresponding aldehydes plus H₂O₂ and ammonia [1]. Dopamine and, to a lesser extent, norepinephrine are oxidized by SSAO, but epinephrine is not. VAP-1 (vascular adhesion protein-1) is a copper-containing SSAO secreted by vascular smooth muscle cells, adipocytes, and endothelial cells with functional monoamine oxidase activity [2]. It may function as a scavenger enzyme to assist MAO, but it is insensitive to MAO inhibitors. The SSAO level in human plasma is very low and exerts a minor influence on catecholamines in *in vitro* incubation [1]. In addition, SSAO overexpression in transgenic mice has had no influence on blood pressure after adrenaline administration, in contrast to the wild type animals [3]. The oxidation process generates harmful products that may be involved in causing atherosclerosis and vascular damage in diabetes. Along with aldehyde and glucose, hydrogen peroxide can modify various proteins to generate advanced glycosylated end products (AGEs), another important factor in the development of atherosclerosis [4]. Elevation of SSAO activity is observed in atherosclerosis, diabetes mellitus and obesity [5-7]. Therefore, as an adhesion molecule and an enzyme, SSAO/VAP-1 can participate in the development of atherosclerosis. It has been reported that subjects with chronic kidney disease have higher serum VAP-1 [8] suggesting a possibility that serum VAP-1 may be excreted by the kidneys. Endothelial dysfunction is highly prevalent in both cardiovascular disease and chronic kidney disease [9, 10]. In addition, recently Li et al. [11] indicated that serum VAP-1 may be a good predictor for cardiovascular mortality. Taking all these data into consideration, the aim of the study was to assess VAP-1 levels and its correlations with endothelial injury markers and renalase, a novel protein probably involved in blood pressure regulation, in prevalent kidney allograft recipients.

Materials and Methods

The studies were performed on 50 prevalent kidney allograft recipients (36 males). Before the transplantation, all the kidney transplant recipients were on renal replacement therapy. The immunosuppressive regimen of prevalent kidney transplant recipients consisted of calcineurin inhibitor, in combination with mycophenolate mofetil/azathioprine and prednisone. All of them maintained sufficient and stable graft function, showed no clinical signs of rejection, no inflammation. All subjects gave informed consent, and the protocol was approved by the Medical University Ethics Committee. Blood was drawn in the morning when patients appeared for routine office assessment after an overnight fast. GFR was estimated using simplified MDRD formula [12] or CKD-EPI equation [13]. Creatinine clearance was estimated using Cockcroft-Gault formula [14]. Complete blood count, urea, phosphate, creatinine, were studied by standard laboratory method in the central laboratory of the hospital. Markers of endothelial cell injury- von Willebrand factor, thrombomodulin and adhesion molecules: ICAM and VCAM were studied using commercially available kits from American Diagnostica, USA, R&D Systems, Quantikine, UK, respectively. Tissue plasminogen activator and its inhibitor-PAI were assayed using kits from Bioopool, Umea, Sweden. High-sensitivity CRP was measured using commercially available assay from American Diagnostica (Greenwich, CT, USA) and IL-6 using commercially available kit from R&D (Abingdon, UK). Renalase was assayed using commercially available kits from Life Sci, Wuhan, China. VAP-1 was assessed using kits from BioVendor, Modrice, Czech Republic. CD40L and CD44 were studied using kits from Bender MedSystem, Vienna, Austria. CD 146 by using kits from BioCytex, Marseille, Visfatin and apelin was assayed by kits from Phoenix, Pharmaceuticals Inc, Belmont, CA, USA, adiponectin and leptin was studied using kits from Linco Research, USA. Healthy volunteers (n = 16) were included in the study to obtain normal ranges for VAP-1. Data were expressed as mean ± SD. The data given were analyzed using Statistica 9.0. computer software (Tulsa, OK, USA). The examination of the distribution normality of variables was done using W Shapiro-Wilk test. The data were also logarithmically transformed to achieve normal distribution, whenever possible. Measurements normally distributed are reported as mean ± SD, non-normally distributed data

Table 1: Clinical and biochemical characteristics of the studied kidney transplant recipients

parameters	statistical value
age (years)	46.16±12.38
time after transplantation (months)	46.20±41.45
hemoglobin (g/dL)	13.46±1.83
creatinine (mg/dL)	1.51±0.56
eGFR by MDRD (ml/min)	57.83±20.73
eGFR by CKD-EPI (ml/min)	59.76±22.45
Creatinine clearance by Cockcroft-Gault (ml/min)	56.16±19.33
Urea (mg/dL)	67.67±30.99
IL-6 (pg/mL)	0.55 (0.04-5.48)
hsCRP (mg/L)	1.16 (0.02-12.76)
ICAM (ng/ml)	264.09±94.98
VCAM (ng/mL)	652.12±518.48
thrombomodulin (ng/ml)	6.95±5.99
vWF (%)	167.40±30.03
CD44 (ng/mL)	73.86±194.11
CD146 (ng/mL)	289.44±112.56
CD40L (ng/mL)	0.95 (0.5-14.0)
Renalase (µg/mL)	6.60±2.78
VAP-1 (ng/mL)	260.11± 139.81
adiponectin (mg/L)	38.95 (9.6-90.99)
leptin (ng/mL)	8.05 (0.8-62.20)
Visfatin (ng/mL)	30.72±17.23
apelin (ng/mL)	50.69±22.99
BMI (kg/m ²)	0.50 (0.04-5.48)
Systolic blood pressure (mm Hg)	134.32±10.92
Diastolic blood pressure (mm Hg)	83.33±6.62
Cholesterol (mg/dL)	189.36±35.99
Triglycerides (mg/dL)	145.33±71.24
Data given are means ± SD, or median and ranges	

Table 2: Correlations between VAP-1 and other parameters studied in kidney transplant recipients

parameters	VAP-1, r and p values
Age (years)	r=0.18, p>0.05
Time after transplantation (months)	r=0.11, p>0.01
Hemoglobin (g/dL)	r=-0.07, p>0.05
Creatinine (mg/dL)	r=0.39, p<0.01
eGFR by MDRD (ml/min)	r=-0.27, p<0.05
eGFR by CKD-EPI (ml/min)	r=-0.27, p<0.05
Creatinine clearance by Cockcroft-Gault (ml/min)	r=-0.35, p<0.01
Urea (mg/dL)	r=0.27, p<0.05
IL-6 (pg/mL)	r=0.20, p>0.05
hsCRP (mg/L)	r=0.28, p<0.05
ICAM (ng/ml)	r=0.11, p>0.05
VCAM (ng/mL)	r=0.11, p>0.05
Thrombomodulin (ng/ml)	r=0.15, p>0.05
vWF (%)	r=0.11, p>0.05
CD44 (ng/mL)	r=0.27, p<0.05
CD146 (ng/mL)	r=0.49, p<0.001
CD40L (ng/mL)	r=0.27, p<0.05
Renalase (µg/mL)	r=-0.34, p<0.05
Adiponectin (mg/L)	r=0.13, p>0.05
Leptin (ng/mL)	r=0.08, p>0.05
Visfatin (ng/mL)	r=-0.07, p>0.05
Apelin (ng/mL)	r=-0.06, p>0.05
BMI (kg/m ²)	r=0.39, p<0.01
Systolic blood pressure (mm Hg)	r=0.16, p>0.05
Diastolic blood pressure (mm Hg)	r=0.14, p>0.05
Cholesterol (mmg/dL)	r=0.24, p>0.05
Triglycerides (mg/dL)	r=-0.06, p>0.05
Cyclosporine A concentration (ng/mL)	r=0.11, p>0.05

are expressed as a median and minimal-maximal value. Spearman or Pearson correlations were evaluated as appropriate with $P<0.05$ considered statistically significant. Multiple regression analysis was used to determine independent factors affecting dependent variable. Factors showing linear correlation with VAP-1 ($p<0.1$) were included in the analysis.

Results

Clinical and biochemical characteristics of the studied kidney transplant recipients are given in the Table 1. The mean serum VAP-1 in Tx was significantly higher comparing to the control group (260.11 ± 139.81 vs 158.34 ± 56.89 ng/mL, $p<0.01$). Diabetic patients ($n=8$) had higher serum VAP-1 than non-diabetic (397.74 ± 102.03 ng/mL vs 232.51 ± 85.27 ng/mL, $p<0.05$) as well as hypertensive patients ($n=30$) when compared to normotensive ones (284.31 ± 124.88 ng/mL vs 197.40 ± 68.96 ng/mL, $p<0.05$). Patients with eGFR below 60 ml/min had higher VAP-1 when compared to those with eGFR ≥ 60 ml/min (275.93 ± 144.63 ng/mL vs 214.68 ± 71.66 ng/mL, $p<0.05$). No difference in VAP-1 was found between patients with and without coronary artery disease ($r=0.29$, $p<0.05$). When we divided kidney allograft recipients according to CKD stages and compared patients with eGFR over 60 ml/min/ 1.73m^2 ($n=24$) relative to eGFR below 60 ml/min/ 1.73m^2 ($n=26$), we found that VAP-1 was significantly higher in subjects with lower eGFR (306.91 ± 176.38 ng/mL vs 222.47 ± 71.02 ng/mL, $p<0.05$, Fig. 1). In kidney transplant recipients VAP-1 correlated several parameters

Fig. 1.

Fig. 2.

(Table2) including renase (Fig. 2). In multiple regression analysis VAP-1 was predicted 80% by serum creatinine (beta value 0.33, $p=0.01$), and CD146 (beta value 43, $p=0.0005$) $F=12.26$, $SE=71.83$ and $p<0.000001$.

Discussion

In our study we showed that VAP-1 is elevated in kidney allograft recipients and predicted by kidney function and a marker of endothelial dysfunction. Even a successful renal transplantation did not restore kidney function to normal and vast majority of kidney transplant recipients have stage 2 or 3 CKD. Chronic kidney disease, particularly at more advanced stages has been associated with the impaired immunity and subclinical inflammation involving cytokines derived from adipose tissue – adipocytokines. Endothelial

cell injury is a common finding in patients following kidney transplantation as we shown previously [9]. In addition, impairment in renal function may also affect the levels of inflammatory molecules, as elevated serum C-reactive protein (CRP) and interleukin-6 levels showed an inverse correlation with creatinine clearance [15]. In our study was found that VAP-1 was significantly higher in kidney transplant recipients when compared to the healthy volunteers. In patients with eGFR over 60 ml/min renalase was significantly lower than in patients with eGFR below 60 ml/min. In the study by Li et al. [8] serum VAP-1 levels were positively associated with the urinary albumin-to-creatinine ratio and inversely correlated with estimated GFR. Patients with CKD stage 2 and stage 3 had significantly higher levels of serum VAP-1 than those without CKD. A high serum VAP-1 level was associated with the presence of CKD after adjustment for age, sex, and smoking. In addition in our population, diabetic (n=6) and hypertensive (n=30) kidney allograft recipients VAP-1 had significantly higher than non-diabetic and normotensive subjects. Li et al. [16] reported previously that serum VAP-1 was higher in subjects with acute and chronic hyperglycemia and with diabetes. Moreover, they also showed for the first time that serum VAP-1 can independently predict 10-year all-cause mortality, cardiovascular mortality, in subjects with type 2 diabetes. In addition, they also noted that the change in serum VAP-1 after glucose challenge was correlated with systemic oxidative stress, AGEs, and carotid intima-medial thickness, which is an index for atherosclerosis [17]. In the experimental studies mice overexpressing VAP-1 in the endothelium have shown increased concentrations of serum AGEs, enhanced leukocyte binding, upregulation of hepatic redox-sensitive proteins, and accelerated atherosclerosis [18]. Endothelial VAP-1 can participate in inflammation by binding granulocytes, lymphocytes, and monocytes, with the aid of SSAO activity [2]. Renalase is supposed to exhibit monoaminooxidase activity. In our previous study, we reported the correlations between renalase and endothelium injury markers, however, the renalase levels were predicted by kidney function [19]. It might be due to the fact that together with a decline in kidney function, endothelial damage was increased. In this study we found a correlation between VAP-1 and renalase as well as between VAP-1 and markers of endothelial cell injury.

Conclusion

VAP-1, elevated in kidney transplant recipients, is predominantly dependent on endothelial damage and kidney function, which deteriorated with time after kidney transplantation. Li et al. [11] suggested even a potential use of VAP-1/SSAO inhibitors or antibodies, currently being tested for their ability to modulate autoimmune disturbances and to treat or prevent cardiovascular diseases, particularly in type 2 diabetes.

Conflict of Interests

The authors state that they have not any conflicts.

Acknowledgements

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